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TRANSMITTAL FORM

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First Named Inventor	Chuan Li
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Remarks

This is the resubmission of the amendment filed on 6/21/04 with signiture and date.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm or Individual name	Chuan Li
Signature	Chuan Li
Date	july 26, 2004

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Typed or printed name	Chuan Li		
Signature	Chuan Li	Date	7/26/04

Amendments to DE NOVO SYNTHESIZED PLASMID, METHODS OF MAKING AND USE THEREOF

Applicant Name: Chuan Li 

Date: July 26, 2004

Application/Control Number: 10/068,664

Art Unit: 1636

a.) Introductory Comments

1. In ABSTRACT, the title "De novo synthesized plasmid, methods of making and use thereof" is deleted.
2. In claim 1, the phrase "without referring an existing plasmid as a template" is added after phrase "A de novo synthesized plasmid" to make the scope of the claim clear.

   In part (a) of claim 1, the term "relevant to" is changed to "causing" according to suggestion.

   In part (b) of claim 1, the term "relevant to" is changed to "causing" according to suggestion.

   In part (b) of claim 1, the term "the" before "selection of a plasmid" is deleted.

   In claim 2, the phrase "the plasmid previously obtained from natural sources" is changed to "an existing plasmid previously obtained from natural sources" to address antecedent basis.

   In claim 2, the term "modified" is changed to "synthesized" to make the claim clear.

   In claim 2, the phrase "as a template" is added to the end of the sentence to make the claim specific.

   In claim 3, the phrase "the plasmid previously obtained from recombinant sources" is changed to "an existing plasmid previously obtained from recombinant sources" to address antecedent basis.

   In claim 3, the term "modified" is changed to "synthesized" to make the claim clear.

In claim 3, the phrase "as a template" is added to the end of the sentence to make the claim specific.

In claim 4, the phrase "wherein the replication origin allows the autonomous plasmid replication in a host cell" is changed to "wherein the replication origin allowing the autonomous plasmid replication in a host cell is from natural or recombinant sources" to make the claim specific. This change also makes claims 2 and 3 clear that the replication origin of the plasmid is from natural or recombinant sources whereas the plasmid is de novo synthesized.

In claim 5, the phrase "wherein the selection marker gene encodes a product indicative of plasmid maintenance in a host cell" is changed to "wherein the selection marker gene encoding a product indicative of plasmid maintenance in a host cell is from natural or recombinant sources" to make the claim specific. This change also makes claims 2 and 3 clear that the selection marker of the plasmid is from natural or recombinant sources whereas the plasmid is de novo synthesized.

3. The applicant believes the claimed invention is not anticipated by Stemmer et al. (cited of record in the information disclosure Statement filed 12 January 2004, now numbered as reference 5) for those skilled in the art at the time the invention was made. The reasons are following:

1). The processes of making the plasmids are different between the teaching of Stemmer et al. and the claimed invention. Stemmer et al. makes the plasmid pUC182Sfi using 134 oligos (page 50, left hand column, last paragraph and right hand column, first paragraph, page 51, first paragraph, and page 51, Fig. 3). They performed three-stage PCRs. Their first PCR has 19 reactions (Fig. 3B) and their second PCR has 11 reactions (Fig. 3C). After third PCR, they need perform restriction digestion and ligation to transform bacteria. Most importantly, they use an existing plasmid as a template. The claimed invention makes the plasmid p4T using only 4 oligos (44, 32, 32, and 32 nucleotides in length respectively, Example 1 of the applied invention). One-stage PCR with two reactions are performed. The PCR products are subjected to a brief Exonuclease III digestion (30 seconds reaction, Li et al. Nucleic Acid Res. 25: 4165-4166 (1997)) and then transform the bacteria without

further restriction digestion or ligation. Most importantly, p4T is de novo synthesized without using an existing plasmid as a template. The process described in the claimed invention has fewer steps and it is significantly simpler and much faster than the process used by Stemmer et al.

2). The resulting plasmids made by Stemmer et al. and by the claimed invention are different. Stemmer et al. synthesized an existing plasmid pUC182Sfi (Stemmer et al. Nature 370, 389-391 (1994)) with known properties using pUC182Sfi as a template. pUC182Sfi can also be readily synthesized by modifying pUC18 using site-directed mutagenesis by those skilled in the art at time the paper was published. In contrast, p4T is a de novo synthesized novel plasmid with unknown properties. It is relatively difficult to make p4T by modifying existing plasmid pACYC177 with current recombinant DNA technology. On the other hand, those skilled in the art would not have the incentive to make the novel plasmid p4T with unpredictable properties. The replication origin of p4T is generated from low copy number plasmid pACYC177; its predicted copy number should be lower than plasmids synthesized from pBR322 and significant lower than the plasmids synthesized from pUC19. However the observed copy number of p4T is higher than or comparable with the plasmids synthesized from pUC19 origin (p3A, Fig. 2) and significant higher than those synthesized from pBR322 origin (p1A, Fig. 2). This unexpected result, which is contradictory to relevant teachings in molecular biology, is not anticipated by Stemmer's teaching or any other prior arts.

3). The uses of the plasmids made by Stemmer et al. and by the claimed invention are different. p182Sfi made by Stemmer et al. (Stemmer et al. Gene 164, 49-53 (1995)) has same uses of the same plasmid made previously (Stemmer et al. Nature 370, 389-391 (1994)). It has similar properties and uses as that of pUC18. In contrast, the replication origin of p4T is synthesized from pACYC177 and its selection marker gene is synthesized from pBR322. The novel plasmid p4T is de novo synthesized without referring either pACYC177, pBR322 or any other plasmids as a template. p4T has higher copy number than pACYC177 and stronger tetracycline resistance than pBR322 (unpublished results). Therefore it has different uses than pACYC177 and pBR322. For example, p4T can be used in the applications that require high copy

number and at same time with the same replication origin as pACYC177 while neither pBR322 nor pACYC177 can be used. Stemmer et al. does not teach the claimed invention.

In conclusion, the disclosed invention uses different process to make plasmid. The de novo synthesized plasmid has novel sequences and novel, sometimes unexpected properties. It is useful in various biomedical applications. It is neither anticipated nor obvious over any prior arts or combination of them. Therefore Claims 1-5 are patentable.